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STUDY OF SHIGELLA VACCINES IN MAN

Richard B. Hornick, M.D.

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Annual Report

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SUMMARY

Studies have continued to evaluate the safety, biologic properties and efficacy of various oral Shigella vaccine candidates. The most recent vaccine candidates employed have been Escherichia coli bearing Shigella group antigens. These vaccine candidates are noteworthy in that they are completely safe with no potential for reversion and they appear to proliferate within the human bowel. In several protocols however involving concomitant challenge of both vaccinated and non-vaccinated volunteers with varied doses of virulent S. flexneri 2a, attack rates were similar and a protective effect of the vaccine strain could not be demonstrated.

Lactulose (beta-1, 4-galactosidofructose), a synthetic derivative of lactose, was evaluated as a possible non-antibiotic therapy for acute Shigella dysentery. Earlier studies had demonstrated the suppressive effect of lactulose on excretion of S. sonnei by a long term carrier. Lactulose was compared with ampicillin and placebo in double-blind fashion in the therapy of acute dysentery. There was no difference in the effects of lactulose and placebo on excretion of shigella, whereas ampicillin abruptly and significantly terminated excretion of shigella in acute dysentery.

Volunteer studies at the Maryland House of Correction, as elsewhere, have been curtailed due to administrative and political difficulties. This has resulted in a temporary decrease in the number and scope of studies undertaken in the past year. It is hoped that this situation will be clarified in the near future allowing a return to a more intensive utilization of the Division's unique facilities at the Maryland House of Correction.

I. INTRODUCTION

During the past fifteen years the Division of Infectious Diseases of the University of Maryland School of Medicine has conducted investigations into the pathogenesis, treatment and control by immunologic means of several enteric diseases. Initial studies were concerned with typhoid fever and potential vaccines for its prevention. In recent years the scope of diseases under investigation has been greatly expanded to include shigella dysentery, enterotoxigenic and invasive Escherichia coli diarrheal disease, acute non-bacterial gastroenteritis of adults (parvovirus) and cholera. For all the above-mentioned diarrheal diseases man is the natural host, reservoir and vector of infection, and the various investigations undertaken have included studies with prison volunteers under strictly controlled conditions in the Research Hospital maintained by the Division at the Maryland House of Correction, Jessup, Maryland.

II. PURPOSE

A. To determine the safety, biologic properties, efficacy and immunization schedules of candidate oral Shigella vaccines. The ultimate goal of these studies is to develop a safe, efficacious, multivalent, easily administered Shigella vaccine for use in high-risk populations such as military personnel, American Indians, and institutionalized children.

B. To investigate further the relative importance, pathogenetic mechanisms, treatment and means for control of diverse pathogens responsible for "non-specific gastroenteritis". These investigations include studies with:

1) Enterotoxigenic E. coli.
2) Invasive E. coli.
3) Parvoviruses.
4) Giardia intestinalis.

C. To investigate both the non-specific and specific defense mechanisms of the human host against the above-mentioned pathogens.

III. BACKGROUND

Shigella infections continue to be an important cause of morbidity in civilian and military populations throughout much of the world. Control of shigella infections is becoming ever more difficult due to the increasing emergence of strains resistant to tetracycline, ampicillin and sulfonamides.

A particularly difficult problem exists with respect to control of infections caused by Shiga's bacillus, Shigella dysenteriae 1. This strain possesses the capability of causing massive epidemics of severe, often fatal, dysentery.[4-8] Since 1968 large-scale epidemics have been encountered in Middle America[4-7] and Bangladesh[8]. The Middle American strain of Shiga's bacillus was resistant to tetracycline, chloramphenicol and sulfonamides, but was sensitive to ampicillin.[4] More recently in Bangladesh S. dysenteriae 1 strains have been recovered with resistance to ampicillin, as well as chloramphenicol, tetracycline and sulfonamides.[8] The newer, less common antibiotics are too expensive to be employed in health service facilities in developing countries such as Bangladesh and Guatemala. Thus, multiply-resistant Shiga bacillus strains continue to pose a grave public health threat for the developing world; similarly, U.S. military personnel in these parts of the world must also be considered at risk. The Middle American Shiga dysentery pandemic was remarkable in that all age groups, particularly working age males, suffered high attack rates.[4-7]

The above observations illustrate that means other than isolation techniques and antibiotics are necessary for the control of shigella infections. In this regard much progress has been made since the early 1960's in development of attenuated strains of shigella for use as oral vaccines. Presently we are involved in evaluation of the "third generation" of oral shigella vaccines. Investigations are continuing in search of safer, more immunogenic vaccines since the first (streptomycin-dependent) and second generation (mutant-hybrid) vaccines are not optimal immunizing agents.

Streptomycin-Dependent Vaccines

These shigella strains developed by Mel and his colleagues have been administered to healthy adults[9,10] and children[11] in Yugoslavia, and adult volunteers[12] and institutionalized children[13] in the U.S.A. They suffer the drawback of apparently requiring multiple doses. Although they proved to be highly efficacious in field trials in endemic areas of Yugoslavia[9-11], they were only moderately efficacious in prison volunteers[12], and in one small controlled study among institutionalized children they gave no benefit.[14] The S. flexneri 2a vaccine is highly stable. In contrast, administration of one particular lyophilized lot of S. sonnei vaccine was associated with recovery of streptomycin-independent vaccine revertants in 18% of vaccinees in one field trial in the U.S.A.[15] The revertants remained non-invasive and did not cause adverse clinical reactions. A subsequent lot of SmD S. sonnei vaccine did not revert in vivo. In this same study evidence of person-to-person transmission of SmD shigella vaccines was obtained.[15]

Mutant-Hybrid Vaccines

These strains were developed by Formal and his co-workers by mating non-invasive shigella colonial mutants with E. coli K-12. The resultant stable mutant-hybrid organisms were two steps removed from virulence. The thrust for development of the mutant-hybrid strains was to create a shigella vaccine that would be as safe and immunogenic as the SmD strains, but that would re-

quire only one or two doses because of its potential to proliferate. Although the mutant-hybrid S. flexneri 2a vaccine was shown to be safe in volunteers[12] and institutionalized children[13], it did not proliferate in the human gut and multiple doses were required. With appearance of pandemic Shiga dysentery in Middle America in 1968-1970, research was begun to develop a mutant-hybrid Shiga vaccine. Because of local conditions in the developing countries of Middle America, any vaccine necessitating more than one or two doses would be impractical. Amongst the candidate vaccine strains that were developed, one strain, 482-2E-1, initially appeared to be an ideal immunizing agent.[16] It emerged as the first mutant-hybrid strain that clearly proliferated in vivo.[16] Most individuals excreted the strain for 7 - 10 days after a single dose, and several excreted for more than 4 weeks. In total 135 men received strain 482-2E-1. In one individual, however, the mutant-hybrid completely dissociated and reverted to a virulent state resulting in classic Shiga dysentery.[16] The revertant organisms were found to be invasive.

IV. RESULTS OF STUDIES OF THE PAST YEAR

A. E. coli Bearing S. flexneri Surface Antigens (E. coli-S. flexneri hybrids)[17]

E. coli-S. flexneri 2a hybrids, representing the third generation of shigella vaccines, have been tested as oral vaccines in volunteers. They have been found to be safe and impart a degree of immunty which approximates that following recovery from active infections. An advantage of the hybrid strain has been greater intestinal proliferation as measured by duration of excretion. It has been hoped that such a hybrid vaccine would be safer than attenuated S. flexneri strains, with no likelihood of reversion to a virulent form, and would require fewer and lower doses to be effective.

In an earlier protocol, volunteers received 2 doses of a new vaccine strain, PGA 142-1-15, separated by one month. This strain is an E. coli 0:8 into which S. flexneri 2a group and type antigens were introduced by means of selective matings utilizing an Hfr$^+$ shigella as the donor.[17] Each dose consisted of approximately 3×10^{10} organisms, and was given in a glass of milk 5 minutes following ingestion of 2 gm $NaHCO_3$. Eight weeks later, 14 vaccinees and 15 controls were challenged with approximately 12,000 virulent "chimp strain" S. flexneri 2a organisms in 45 cc milk. No significant differences were noted with regard to case rates, infection rates, or duration of excretion of organisms.

There are several possible explanations for the lack of efficacy: 1) The vaccine is inherently ineffective; 2) it was given in insufficient dosage; 3) the challenge dose was excessive and overwhelmed the protective effect of the vaccine. There is reason for believing the latter explanation. In previous studies at Jessup, a challenge dose of 5000 or more organisms has resulted in a case rate consistently around 60%, whereas 180 organisms gave an $I.D._{25}$.[12] Data from community surveys are few, but in one such study[18] there were 9.1 asymptomatic or convalescent carriers for each case. This would suggest that the natural infecting dose might constitute approximately an $I.D._{10}$.

Patients

To test this latter possibility, 58 healthy volunteers were given 3 doses of the vaccine strain, PGA 142-1-15 at one week intervals. The dose given was $2.3 - 3.1 \times 10^{10}$ organisms in 45 ml of milk, 5 minutes after ingestion of 2 gm $NaHCO_3$. Forty-eight men received all 3 doses, 8 received 2 doses and 2 received one dose. There were no adverse reactions.

All men submitting an adequate number of stool specimens excreted the vaccine organism on multiple occasions. The duration of excretion of the organism following the first 2 doses could not totally be assessed due to the one week interval between doses. However, the minimum average duration after the first dose was 5.4 days, after the second 4.9 days, and after the third 6.1 days. Thus, there was no discernible diminution in excretion with successive doses. There was no appreciable difference in recovery of the organism from stool specimens or from rectal swabs. There were no adverse reactions to the vaccine.

The ability of the vaccine organism to be transmitted from person-to-person was investigated in a single blind fashion by placing 6 vaccinees and 5 men receiving placebo in the hospital ward for 4 weeks. No transmission was detected during their hospital stay. However, 3 days after discharge, one control had a single positive stool culture. He shared a cell with one of the vaccinees whose stool had last been positive 8 days before.

On May 22, 1974, four weeks after the last dose of vaccine, 43 vaccinees and 36 controls received a challenge dose of 138 organisms of the virulent S. flexneri 2a "chimp strain". Of the total vaccinees, 39 had received 3 vaccine doses, and 4 had received 2 doses. Thirty-five men became ill with shigellosis, 20/43 (47%) of vaccinees and 15/36 (42%) of controls. The treatment regimen was given to alternate hospital admissions as follows: either ampicillin 3 gm p.o. stat or ampicillin 250 mg p.o. qid for 3 days. Seven of 20 (35%) vaccinees and 5 of 15 (33%) controls suffered clinical relapses. Four of the relapses had been treated with the stat dose of ampicillin, and 7 with the 3 day regimen. One relapse was treated with chloramphenicol due to a history of penicillin and tetracycline allergy.

Bacteriology results are as yet incomplete. To date, 15/20 (75%) of ill vaccinees and 12/15 (80%) of ill controls have had one or more positive cultures. Only 1/23 of well vaccinees and 0/22 well controls have had positive cultures. Contrary to the recovery rate of the vaccine organisms, the virulent "chimp strain" was recovered in 21% of stool specimens but only 5% of rectal swabs. There was no difference in the recovery rates among vaccinees or controls.

The analysis of serum specimens for serologic titers, liver function tests, and serum iron has not been completed.

Other Studies

Earlier, two other flexneri vaccine candidate strains were safety tested in small groups of

men. They were prepared in a fashion identical to that of the strain PGA 142-1-15 described above. The results of testing is summarized below:

Shigella serotype	Approximate dose	Number of Men	Number Positive	Average days Positive
3b	10^6	6	0	-
3b	10^8	5	3	5.0
3b	10^{10}	5	5	5.8
1a	10^6	5	2	3.5

No adverse effects were noted in the volunteers. However, because of the lack of encouraging results obtained with challenge studies involving the S. flexneri 2a strain, no further work with these other strains is contemplated at present.

B. Lactulose

Previous observations on the pathophysiology of shigella infections in man and experimental animals were applied in the search for a non-antibiotic therapeutic agent for use in human shigellosis. The work of Hentges, et al[19-23] and others[24-27] have clearly shown the antagonism between normal colonic flora and shigella and salmonella enteropathogens. This antagonism is apparently mediated by the striking inhibitory effects of short chain organic acids (lactic, acetic, formic) which are end products of disaccharide metabolism of normal colonic flora, and which are deleterious to shigella.[20, 28, 29] The suppressive effect of these short-chain acids is pH dependent[21, 28, 29]; the lower the pH, the greater their toxicity. This suggests that it is the undissociated molecules which are most active.[21, 28, 29]

Earlier studies done at the University of Maryland (H.L. DuPont and R.B. Hornick, unpublished observations) have demonstrated an inverse relation between intestinal concentrations of short chain fatty acids (acetic, butyric and propionic) and excretion of shigella in the course of human infection.

Lactulose (beta-1, 4-galactos dofructose) is a synthetic derivative of lactose containing one molecule of galactose and one of fructose.[30] It is marketed in Europe as a 50% W/W syrup ("Duphalac", Philips-Duphar) and is widely used as a laxative. It has been used in the U.S.A. and elsewhere experimentally in the treatment of hepatic encephalopathy.[30] Lactulose

is not absorbed by the human intestine, nor is it hydrolyzed by human intestinal enzymes.[30, 31] Lactulose passes intact to the colon where normal saccharolytic flora (predominantly lactobacilli and Bacteroides) actively metabolize it.[30] The end products of this bacterial metabolism are short chain fatty acids which are lethal to shigella. Shigella, a non-lactose fermenter, is unable to hydrolyze lactulose.

We first employed lactulose therapeutically in an attempt to eradicate shigella infection in a long-term asymptomatic shigella carrier in whom all previous antibiotic therapy had failed.[32] Two 14 day courses of lactulose were administered three months apart. Lactulose had a striking effect in decreasing excretion of shigella. However, in both instances excretion returned to pre-treatment levels upon cessation of therapy.[32]

Lactulose was next employed in the therapy of acute shigella dysentery. Nine adult male volunteers who developed acute shigellosis in the course of an efficacy test of a S. flexneri 2a vaccine candidate were randomly allocated oral ampicillin (500 mg q6h), placebo, or or lactulose (35 ml q6h) in double blind fashion. In addition one volunteer, known to be allergic to penicillin was given lactulose in single blind fashion. Ampicillin, placebo and lactulose were given in the same volumes and were prepared by a pharmacist to be similar in color, consistency and taste. The severity of disease was similar in all three groups undergoing therapy. Bacteriologic response was monitored by daily rectal swab cultures for 5 consecutive days.

As in previous experience ampicillin was highly effective and all three men who received this antibiotic had negative cultures by the 4th day after therapy was begun. In contrast all of the men on placebo and all the men receiving lactulose were still excreting shigella. Tetracycline therapy promptly arrested shigella excretion in these men. These data are tabulated in Table 1.

These results suggest that lactulose may have a role in the therapy of shigella carriers. However, there is no indication for further trials with lactulose in treatment of acute dysentery. The difference in the effects of lactulose in these two situations is presumably due to the fact that shigella is probably confined to the bowel lumen in the asymptomatic shigella carrier state whereas in acute dysentery the organism is proliferating within the colonic mucosal cells and lamina propria.

TABLE 1.

Effects of Lactulose, Ampicillin and Placebo on Excretion of

Shigella flexneri 2a During Acute Dysentery

Treatment Group	Number of Men	Number of men with positive cultures: Days after commencement of therapy: 0	3	4
Ampicillin	3	3	1	0
Lactulose	4	4	4	4
Placebo	3	3	3	3

Ampicillin vs. Placebo, p = .05*

Ampicillin vs. Lactulose, p = .03*

Lactulose vs. Placebo, *no significant difference**

*Fisher's exact test

REFERENCES

1. Ross, S., Controni, G., Khan, W.: Resistance of Shigellae to Ampicillin and Other Antibiotics. J.A.M.A. 221:45, 1972.

2. Tilton, R.C., Corcoran, L., Newberg, L., Sedgwick, A.K.: Ampicillin-Resistant Shigella sonnei. J.A.M.A. 222:487, 1972.

3. Lerman, S.J., Waller, J.M., Simms, D.H.: Resistance of Shigellae to Ampicillin and Other Antibiotics: South Bronx, New York (1971 and 1972). J. Pediatr. 83: 500, 1973.

4. Mata, L.J., Gangarosa, E.J., Caceres, A., et al: Epidemic Shiga bacillus Dysentery in Central America. I. Etiologic Investigations in Guatemala, 1969. J. Infect. Dis. 122:170, 1970.

5. Gangarosa, E.J., Perera, D.R., Mata, L.J., et al: Epidemic Shiga bacillus Dysentery in Central America. II. Epidemiologic Studies in 1969. J. Infect. Dis. 122:181, 1970.

6. Mendizabal-Morris, C.A., Mata, L.J., Gangarosa, E.J., et al: Epidemic Shiga bacillus Dysentery in Central America. Derivation of the Epidemic and Its Progression in Guatemala, 1968-1969. Am. J. Trop. Med. and Hyg. 20:927, 1971.

7. Reller, L.B., Navarro Rivas, E., Masferrer, R., et al: Epidemic Shiga bacillus Dysentery in Central America. Evolution of the Outbreak in El Salvador. Am. J. Trop. Med. and Hyg. 20:934, 1971.

8. Rahaman, M.M., Huq, I., Dey, C.R., et al: Ampicillin-Resistant Shiga bacillus in Bangladesh. Lancet 1:406, 1974.

9. Mel, D.M., Terzin, A.L., and Vuksic, L.: Studies on Vaccination against Bacillary Dysentery. III. Effective Oral Immunization against Shigella flexneri 2a in a Field Trial. Bull. WHO 32:647, 1965.

10. Mel, D.M., Arsic, B.L., Nikolic, B.D., et al: Studies on Vaccination against Bacillary Dysentery. IV. Oral Immunization with Live Monotypic and Combined Vaccines. Bull. WHO 39:375, 1968.

11. Mel, D.M., Gangarosa, E.J., Radovanovic, M.D., et al: Studies on Vaccination against Dysentery. VI. Protection of Children with Oral Immunization Using Streptomycin-Dependent Shigella Strains. Bull. WHO 45:457, 1971.

12. DuPont, H.L., Hornick, R.B., Snyder, M.J., et al: Immunity in Shigellosis. II. Protection Induced by Oral Live Vaccine or Primary Infection. J. Infect. Dis. 125:12, 1972.

13. Levine, M.M., DuPont, H.L., Gangarosa, E.J., et al: Shigellosis in Custodial Institutions. II. Clinical, Immunologic and Bacteriologic Response of Institutionalized Children to Oral Attenuated Shigella Vaccines. Am. J. Epidemiol. 96:40, 1972.

14. Levine, M.M., Gangarosa, E.J., Werner, M., et al: Shigellosis in Custodial Institutions. III. Prospective Clinical and Bacteriologic Surveillance of Children Vaccinated with Oral Attenuated Shigella Vaccines. J. Pediatr. 84:803, 1974.

15. Levine, M.M., Gangarosa, E.J., Barrow, W.B., et al: Shigellosis in Custodial Institutions. IV. In vivo Stability and Transmissability of Oral Attenuated Streptomycin-Dependent Shigella Vaccines. Manuscript submitted for publication.

16. Levine, M.M., DuPont, H.L., Formal, S.B., et al: Pathogenesis of Shigella dysenteriae 1 (Shiga) Dysentery. J. Infect. Dis. 127:261, 1973.

17. Formal, S.B., Gemski, P. Jr., Baron, L., et al: Genetic Transfer of Shigella flexneri Antigens to Escherichia coli K-12. Infect. Immun. 3:279, 1970.

18. Watt, J., Hardy, A.V.: Studies of the Acute Diarrheal Diseases. XIII. Cultural Surveys of Normal Population Groups. Public Health Reports 60:261, 1945.

19. Hentges, D.J., Freter, R.: In vivo and In vitro Antagonism of Intestinal Bacteria against Shigella flexneri. I. Correlation Between Various Tests. J. Infect. Dis. 110:30, 1962.

20. Hentges, D.J.: Inhibition of Shigella flexneri by the Normal Intestinal Flora. II. Mechanisms of Inhibition of Coliform Organisms. J. Bacteriol. 97:513, 1969.

21. Hentges, D.J., Maier, B.R.: Inhibition of Shigella flexneri by the Normal Intestinal Flora. III. Interactions with Bacteroides fragilis Strains In vitro. Infect. Immun. 2:364, 1970.

22. Maier, B.R., Hentges, D.J.: Experimental Shigella Infections in Laboratory Animals. I. Antagonism by Normal Flora Components in Ghotobiatri Mice. Infect. Immun. 6:168, 1972.

23. Maier, B.R., Onderdonk, A.B., Baskett, R.C., Hentges, D.J.: Shigella, Indigenous Flora Interactions in Mice. Am. J. Clin. Nutr. 25:1433, 1972.

24. Halbert, S.P.: The Antagonism of Coliform Bacteria against Shigellae. J. Immunol. 58:153, 1948.

25. Freter, R.: In vivo and In vitro Antagonism of Intestinal Bacteria against Shigella flexneri. II. The Inhibitory Mechanism. J. Infect. Dis. 110:38, 1962.

26. Freter, R.: Experimental Enteric Shigella and Vibrio Infections in Mice and Guinea Pigs. J. Exp. Med. 104:411, 1956.

27. Nakamura, M.: Alteration of Shigella Pathogenicity by Other Bacteria. Am. J. Clin. Nutr. 25:1441, 1972.

28. Hentges, D.J.: Influence of pH on the Inhibitory Activity of Formic Acid and Acetic Acids for Shigella. J. Bacteriol. 93:2029, 1967.

29. Baskett, R.C., Hentges, D.J.: Shigella flexneri Inhibition by Acetic Acid. Infect. Immun. 8:91, 1973.

30. Avery, G.S., Davies, E.F., Brogden, R.N.: Lactulose: A Review of Its Therapeutic and Pharmacologic Properties with Particular Reference to Ammonia Metabolism and Its Mode of Action in Portal Systemic Encephalopathy. Drugs 4:7, 1972.

31. Kahlquist, A., Grybowski, J.: Inability of Human Small Intestinal Lactose to Hydrolyze Lactulose. Biochem. et Biophys. Acta 110:635, 1965.

32. Levine, M.M., DuPont, H.L., Khodabandelou, M., Hornick, R.B.: Long-Term Shigella Carrier State. New Engl. J. Med. 288:1169, 1973.

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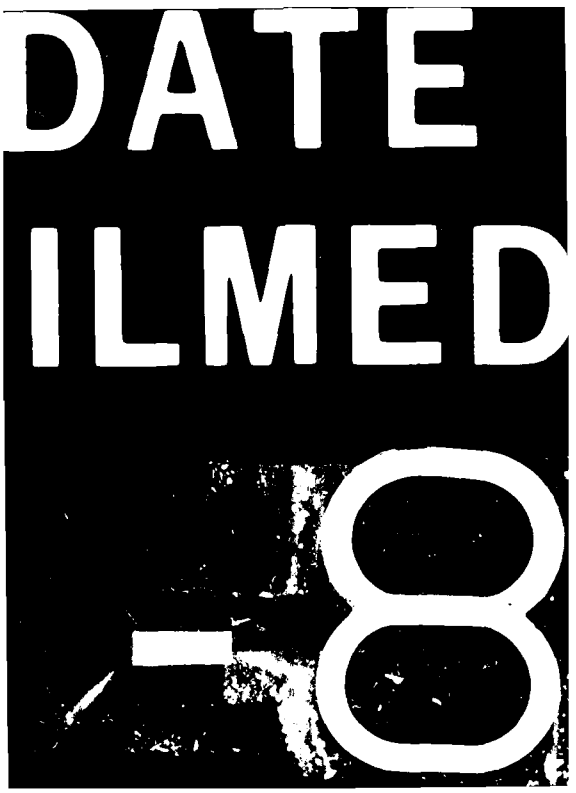
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